UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

April 5, 2010

MEMORANDUM

Subject: Efficacy Review for CARB;
EPA Reg. No. 67619-21; DP Barcode: D373906

From: Marcie Tidd, Microbiologist Marcie Tidd 4/2/10
Product Science Branch
Antimicrobials Division (7510P)

Thru: Tajah Blackburn, Team Leader
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To: Jacqueline McFarlane 34 / Killian Swift
Regulatory Management Branch II
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Applicant: Clorox Professional Products Company
c/o PS&RC; P.O. Box 493
Pleasanton, CA 94566

Formulation from the Label:

Active Ingredient(s)	% by wt.
Octyl decyl dimethyl ammonium chloride	0.1890%
Dioctyl dimethyl ammonium chloride	0.0945%
Didecyl dimethyl ammonium chloride	0.0945%
Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chlorides	0.2520%
Ethanol	58.0600%
Other Ingredients	41.3100%
Total	100.0000%

I. BACKGROUND

The product, Carb (Reg. No. 67619-21), is an Agency-approved disinfectant (bactericide, fungicide, virucide), and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food service, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add new claims for effectiveness as a disinfectant against additional microorganisms, including *Mycobacterium* bovis BCG. Studies were conducted at MICROBIOTEST, located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant to EPA (dated January 15, 2010), Form 8570-1 (Application for Pesticide), Form 8570-4 (Confidential Statement of Formula), Form 8570-34 (Certification with Respect to Citation of Data), Form 8570-35 (Data Matrix), forty nine studies (MRID 479589-01 through 479589-49), Statements of No Data Confidentiality Claims for all forty nine studies, and the proposed label.

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: anesthesia machines, animal equipment, aphaeresis machines, appliance exteriors, appliance knobs, autoclaves, bathtubs, bed frames, bed rails, bedpans, blinds, blood pressure cuffs, cabinet knobs, cabinets, cages, carts, charging stations, cooler exteriors, computer keyboards, computer peripherals, computer screens, counter tops, cords, counters, cupboards, diagnostic equipment, diaper changing stations, diaper pails, dictating equipment, dish racks, docking stations, door handles, doorknobs, drain boards, drawer pulls, elevator buttons, endodontic equipment, equipment surfaces, exercise machines, exhaust fans, faucets, fax machines, feed rack exteriors, fixtures, floors, food cases, food trays, furniture, garbage cans, grocery carts, gurneys, gymnastic equipment, hampers, hand rails, handles, head sets, hospital equipment, IV poles and stands, keyboards, light switches, lights, lockers, mattress covers, medical equipment surfaces, mobile workstations, monitors, mouse pads, office machinery, outdoor furniture, outdoor grill surfaces, paddles, patient monitoring equipment, patient support and delivery equipment, patio furniture, pens, personal protective safety equipment, playground equipment, play structures, privacy curtain edges, railings, recycling bins, remote controls, scales, seats, shelves, shower curtains and doors, shower stalls, signs, sinks, sneeze guards, spine backboards, sports equipment, stalls, stethoscopes, stools, storage bins, stretchers, telecommunication equipment, telephones, tires, toilet handholds, toilets, tools, towel dispensers, toy boxes, trays, trash cans, ultrasound transducers, urinals, vanity tops, veterinary equipment, walkers, wallpaper, walls, wash basins, waste baskets, watering appliance exteriors, wheelchairs, whirlpool tubs, windowsills, work benches, and wrestling mats. The proposed label indicates that the product may be used on hard, non-porous surfaces including: baked enamel, crystal, finished hardwood, Formica, glazed ceramic, glazed porcelain, glazed tiles, laminate, linoleum, Marlite, metal (e.g., chrome, stainless steel), painted surfaces, painted woodwork, plastic (e.g., vinyl), Plexiglas, sealed fiberglass, and synthetic marble.

Directions on the proposed label provide the following information regarding use of the product as a disinfectant: Spray 6-10 inches from pre-cleaned surface for 3-4 seconds or until

wet. Surfaces must remain wet for 10 minutes. A potable water rinse is required for food contact surfaces.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using the AOAC Germicidal Spray Products as Disinfectants Method)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Germicidal Spray Products as Disinfectants Method contains procedures for testing fungicidal activity. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method)

Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

IV. SUMMARY OF SUBMITTED STUDIES

1. MRID 479589-01 "AOAC Germicidal Spray Test Supplemental," Test Organism: Community-Associated Methicillin-Resistant *Staphylococcus aureus*, Genotype 400 (CA-MRSA 400); Clinical Isolate 08005, for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-479.

This study was conducted against Community-Associated Methicillin-Resistant *Staphylococcus aureus* Genotype 400 (Clinical Isolate 08005; received from the University of Louisville Hospital, Louisville, KY). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, according to Microbiotest protocol 320.5.09.16.08. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch, with a treated area of 1 inch x 1 inch) per product lot were inoculated with 0.01-0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following

the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Genotype 400 was verified on a representative culture. An individual Mueller Hinton Agar plate was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Genotype 400 to oxacillin. See pages 9 and 15 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

2. MRID 479579-02 "AOAC Germicidal Spray Test Supplemental," Test Organism: Multidrug-Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503) for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-482.

This study was conducted against Multidrug-Resistant *Klebsiella pneumoniae* (ATCC 51503). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, according to Microbiotest protocol 320.8.09.16.08. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.01-0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 20-40 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Multidrug-Resistant *Klebsiella pneumoniae* (ATCC 51503) was verified on a representative culture. An individual Mueller Hinton Agar plate was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Multidrug-Resistant *Klebsiella pneumoniae* (ATCC 51503) to ceftazidime. See pages 9 and 15 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

3. MRID 479579-03 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Streptococcus pyogenes* (ATCC 19615), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-484.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, according to Microbiotest protocol 320.10.09.16.08. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.01-0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 20-40 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C under candle jar conditions (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed.

4. MRID 479579-04 "Virucidal Effectiveness Test, Human Immunodeficiency Virus Type 1" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – August 14, 2009. Laboratory Project Identification Number 320-495.

This study was conducted against Human immunodeficiency virus type 1 (strain not specified; obtained from ZeptoMetrix Corporation), using C8166 cells (obtained from the University of Pennsylvania) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Human Immunodeficiency virus Type 1," dated October 10, 2008

(copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 35 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. C8166 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 9-12 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

5. MRID 479579-05 "Amended Final Report, Virucidal Effectiveness Test, Respiratory Syncytial Virus, ATCC VR-26" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – January 8, 2009. Amended report date – May 29, 2009. Laboratory Project Identification Number 320-497.

This study was conducted against Respiratory syncytial virus (strain not specified; ATCC VR-26), using HeLa cells (obtained from Diagnostic Hybrids) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Respiratory Syncytial Virus," dated October 10, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 21°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in DMEM with 5% fetal bovine serum. HeLa cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 3-5 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The initial report was amended to "exclude the data obtained from large volume inoculation" and clarify that "all titers were calculated using the Spearman-Karber method."

Note: Protocol deviations/amendments reported in the study were reviewed.

6. MRID 479579-06 "Virucidal Effectiveness Test, SARS-associated Coronavirus" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – August 7, 2009. Laboratory Project Identification Number 320-498.

This study was conducted against SARS-associated coronavirus (CDC strain 200300592; obtained from ZeptoMetrix Corporation), using Vero E6 cells (ATCC CRL-1586) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test SARS-associated Coronavirus," dated October 10, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. Vero E6 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-9 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The laboratory reported a failed study set up on December 11, 2008. In that study, no virus was detected in the plate recovery control. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on December 24, 2008. In that study, the organic load was found to be only 2% serum. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on January 14, 2009. See page 8 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

7. MRID 479579-07 "Confirmatory Virucidal Effectiveness Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Carb, F2008.0034, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-500.

This confirmatory study, under the direction of Study Director Zheng Chen, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Confirmatory Virucidal Effectiveness Test Duck Hepatitis B virus (Surrogate for Human Hepatitis B virus)," dated October 10, 2008 (copy provided). The product was received ready-to-use. The viral stock contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Five replicates per product lot were tested. For each product lot, separate dried virus films were sprayed with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in L-15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed and returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

8. MRID 479579-08 "Virucidal Effectiveness Test, Coxsackievirus B3" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – August 14, 2009. Laboratory Project Identification Number 320-507.

This study was conducted against Coxsackievirus B3 (ATCC VR-30), using LLC-MK2 cells (ATCC CCL-7.1) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Coxsackievirus B3," dated November 1, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 25 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20°C. Five replicates per product lot were tested.

Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. LLC-MK2 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 2-5 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

9. MRID 479579-09 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Burkholderia cepacia* (ATCC 25416), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-518.

This study was conducted against *Burkholderia cepacia* (ATCC 25416). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, as recorded in Microbiotest protocol 320.3.01.17.09. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80, 1% Tamol, and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: The laboratory reported a failed study set up on March 11, 2009. In that study, carrier count control results did not meet the criteria for a valid test. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated. See page 8 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

10. MRID 479579-10 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Corynebacterium diphtheriae* (ATCC 11913), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-520.

This study was conducted against *Corynebacterium diphtheriae* (ATCC 11913). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 as recorded in Microbiotest protocol 320.5.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations appear were reviewed.

11. MRID 479579-11 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Escherichia coli* (ATCC 11229), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-521.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.6.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 36 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers

were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

12. MRID 479579-12 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Enterobacter cloacae* (ATCC 35549), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-523.

This study was conducted against *Enterobacter cloacae* (ATCC 35549). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 as recorded in Microbiotest protocol 320.8.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 36 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

13. MRID 479579-13 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Klebsiella oxytoca* (ATCC 43165), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-525.

This study was conducted against *Klebsiella oxytoca* (ATCC 43165). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods

of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.10.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

14. MRID 479579-14 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Listeria monocytogenes* (ATCC 19111), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-529.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.14.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Brain Heart Infusion Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

15. MRID 479579-15 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Proteus mirabilis* (ATCC 7002), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-530.

This study was conducted against *Proteus mirabilis* (ATCC 7002). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.15.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

16. MRID 479579-16 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Proteus vulgaris* (ATCC 27973), for Carb, F2008.0034, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-531.

This study was conducted against *Proteus vulgaris* (ATCC 27973). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.16a.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to

neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

17. MRID 479579-17 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Salmonella enterica; serovar Paratyphi B* (ATCC 8759), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-534.

This study was conducted against *Salmonella enterica; serovar Paratyphi B* (ATCC 8759). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as described in Microbiotest protocol 320.19.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

18. MRID 479579-18 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Salmonella typhi* (ATCC 6539), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-535.

This study was conducted against *Salmonella typhi* (ATCC 6539). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC

Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as described in Microbiotest protocol 320.20.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 36 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations appear were reviewed.

19. MRID 479579-19 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Serratia marcescens* (ATCC 14756), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-536.

This study was conducted against *Serratia marcescens* (ATCC 14756). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as described in Microbiotest protocol 320.21.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

20. MRID 479579-20 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Shigella dysenteriae* (ATCC 13313), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-537.

This study was conducted against *Shigella dysenteriae* (ATCC 13313). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as described in Microbiotest protocol 320.22.01.17.09. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

21. MRID 479579-21 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Stenotrophomonas maltophilia* (ATCC 13637), for Carb, F2008.0034, by Felicia L. Sellers. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-539.

This study was conducted against *Stenotrophomonas maltophilia* (ATCC 13637). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as described in Microbiotest protocol 320.24.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at

a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 30±2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

22. MRID 479579-22 "Virucidal Effectiveness Test, Adenovirus Type 14, ATCC VR-15" for Carb, F2008.0034, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – August 4, 2009. Laboratory Project Identification Number 320-549.

This study was conducted against Adenovirus type 14 (strain not specified; ATCC VR-15), using A549 cells (ATCC CCL-185) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Adenovirus Type 14," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Dulbecco's Modified Eagle's Medium with 5% fetal bovine serum. A549 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 9-12 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

23. MRID 479579-23 "Virucidal Effectiveness Test, Hepatitis A virus" for Carb, F2008.0034, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 5, 2009. Laboratory Project Identification Number 320-553.

This study was conducted against Hepatitis A virus (strain not specified; obtained from the University of Ottawa), using FRhK-4 cells (obtained from the University of Ottawa) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Hepatitis A virus," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 13 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20-21°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in DMEM with 5% fetal bovine serum. FRhK-4 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 16-20 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

24. MRID 479579-24 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Candida albicans* (ATCC 10231), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 11, 2009. Laboratory Project Identification Number 320-485.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.11.09.16.08. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 1 minute at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to

neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: Repeat testing was performed using one batch (i.e., Lot No. 2008-eg-08) on December 24, 2008.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

25. MRID 479579-25 "AOAC Tuberculocidal Activity of a Germicidal Spray," Test Organism: *Mycobacterium bovis* BCG, for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 22, 2009. Laboratory Project Identification Number 320-486.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.12.09.16.08. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot per contact time were inoculated with 0.02 mL of a 21-25 day old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 5 or 9.5 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to individual tubes of 20 mL of Modified Proskauer-Beck Medium with 7% Polysorbate 80 and 1% Lecithin (which differs from the AOAC method specification of using horse serum to neutralize). The tubes containing neutralizer were shaken thoroughly after addition of the carrier, as specified in the AOAC method. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. Subculture tubes were also shaken thoroughly. All tubes used for secondary transfers were incubated for 60 days at 37±2°C. The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism. Neutralizer effectiveness was conducted at the longest contact time.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, product neutralization, and subculture incubation. The deviations were reviewed.

26. MRID 479579-26 "Initial Virucidal Effectiveness Test, Feline Calicivirus (Surrogate for Human Norovirus)" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-492.

This study, under the direction of Study Director S. Steve Zhou, was conducted against Feline calicivirus (strain not specified; ATCC VR-782), using CrFK cells (ATCC CCL-94) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Initial Virucidal Effectiveness Test Feline calicivirus (Surrogate for Human Norovirus)," dated October 10, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20°C. Following exposure, the plates were neutralized with an equal volume of newborn calf serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially with RPMI 1640 with 5% newborn calf serum. CrFK cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

27. MRID 479579-27 "Initial Virucidal Effectiveness Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – September 1, 2009. Laboratory Project Identification Number 320-493.

This study, under the direction of Study Director S. Steve Zhou, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Initial Virucidal Effectiveness Test Duck Hepatitis B virus (Surrogate for Human Hepatitis B virus)," dated October 10, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 25 minutes at

ambient temperature. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially with L-15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed and returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were.

28. MRID 479579-28 "Confirmatory Virucidal Effectiveness Test, Feline Calicivirus (Surrogate for Human Norovirus)" for Carb, F2008.0034, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – October 7, 2009. Laboratory Project Identification Number 320-499.

This confirmatory study, under the direction of Study Director Salimatu Jibril, was conducted against Feline calicivirus (strain not specified; ATCC VR-782), using CrFK cells (ATCC CCL-94) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Confirmatory Virucidal Effectiveness Test Feline calicivirus (Surrogate for Human Norovirus)," dated October 10, 2008 (copy provided). The product was received ready-to-use. The viral stock contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 17 minutes at ambient temperature. Five replicates per product lot were tested. For each product lot, separate dried virus films were sprayed with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 19-21°C. Following exposure, the plates were neutralized with an equal volume of newborn calf serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. CrFK cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

29. MRID 479579-29 "Virucidal Effectiveness Test, Rotavirus, ATCC VR-899" for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-505.

This study was conducted against Rotavirus (strain not specified; ATCC VR-899), using MA-104 cells (obtained from Charles River Laboratories) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Rotavirus," dated November 1, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 10 minutes at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of Minimum Essential Medium with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 1.0 µg/mL Trypsin. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

30. MRID 479579-30 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Campylobacter jejuni* (ATCC 29428), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 22, 2009. Laboratory Project Identification Number 320-519.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.4.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual

carriers were transferred to tubes containing Brucella Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C under candle jar conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

31. MRID 479579-31 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Enterobacter aerogenes* (ATCC 13048), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-522.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as recorded in Microbiotest protocol 320.7.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 36 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

32. MRID 479579-32 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Enterococcus faecalis* (ATCC 29212), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 22, 2009. Laboratory Project Identification Number 320-524.

This study was conducted against *Enterococcus faecalis* (ATCC 29212). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the

AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 as recorded in Microbiotest protocol 320.9.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

33. MRID 479579-33 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Klebsiella pneumoniae* (ATCC 4352), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 11, 2009. Laboratory Project Identification Number 320-526.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.11.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

34. MRID 479579-34 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Klebsiella pneumoniae* ESBL (ATCC 700603), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-527.

This study was conducted against *Klebsiella pneumoniae* ESBL (ATCC 700603). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.12.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 40 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Antibiotic resistance of *Klebsiella pneumoniae* ESBL (ATCC 700603) was verified on a representative culture. An individual Mueller Hinton Agar plate was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk of either ampicillin or ceftazidime was added to the center of the plate. The plate was incubated and, following incubation, the zones of inhibition were measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of *Klebsiella pneumoniae* ESBL (ATCC 700603) to ampicillin. The measured zone of inhibition (i.e., 11 mm) confirmed antibiotic resistance of *Klebsiella pneumoniae* ESBL (ATCC 700603) to ceftazidime. See pages 9 and 22 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

35. MRID 479579-35 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Legionella pneumophila* (ATCC 33153), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-528.

This study was conducted against *Legionella pneumophila* (ATCC 33153). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.13.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 32 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Charcoal Yeast Extraction Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C under candle jar conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

36. MRID 479579-36 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Pseudomonas putida* (ATCC 12633), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-532.

This study was conducted against *Pseudomonas putida* (ATCC 12633). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.17.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 35 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1%

Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 30±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

37. MRID 479579-37 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Salmonella enteritidis* (ATCC 13076), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-533.

This study was conducted against *Salmonella enteritidis* (ATCC 13076). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.18.01.17.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

38. MRID 479579-38 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Streptococcus pneumoniae* (ATCC 33400), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-540.

This study was conducted against *Streptococcus pneumoniae* (ATCC 33400). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.25.01.17.09.

The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 32 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80, 1% Lecithin, and 5% defibrinated sheep's blood to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: The laboratory reported a failed study set up on March 19, 2009. In that study, carrier counts were low. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated. See page 8 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

39. MRID 479579-39 "Virucidal Effectiveness Test, Hantavirus (Prospect Hill Virus), University of Western Ontario" for Carb, F2008.0034, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – September 16, 2009. Laboratory Project Identification Number 320-547.

This study was conducted against Hantavirus (Prospect Hill Virus; obtained from the University of Western Ontario), using Vero E6 cells (ATCC CRL-1586) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Hantavirus (Prospect Hill virus)," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. Vero E6 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The inoculum was allowed to adsorb for 20-30 hours at 36±2°C with 5±1% CO_2. The cultures were incubated for a total of 10-14 days at 36±2°C with 5±1% CO_2. Following incubation, the cultures were assayed for the presence of

infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

40. MRID 479579-40 "Virucidal Effectiveness Test, Adenovirus Type 2, ATCC VR-846," for Carb, F2008.0034, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – September 1, 2009. Laboratory Project Identification Number 320-548.

This study was conducted against Adenovirus type 2 (strain not specified; ATCC VR-846), using A549 cells (ATCC CCL-185) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Adenovirus Type 2," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 9 inches from the carrier surface. The carriers were allowed to remain wet for 10 minutes at 21°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 0.5% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in DMEM with 5% fetal bovine serum. A549 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 11-14 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

41. MRID 479579-41 "Virucidal Effectiveness Test, Echovirus Type 12, ATCC VR-42" for Carb, F2008.0034, by Tien V. Mai. Study conducted at MICROBIOTEST. Study completion date – September 17, 2009. Laboratory Project Identification Number 320-551.

This study was conducted against Echovirus type 12 (strain not specified; ATCC VR-42), using LLC-MK2 cells (ATCC CCL-7.1) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Echovirus Type 12," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30

minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 6 inches from the carrier surface. The carriers were allowed to remain wet for 10 minutes at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 0.5% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. LLC-MK2 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 3-5 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of *Spearman Karber*.

Note: Protocol deviations/amendments reported in the study were reviewed.

42. MRID 479579-42 "Virucidal Effectiveness Test Herpes Simplex virus Type 1, ATCC VR-260" for Carb, F2008.0034, by Tien V. Mai. Study conducted at MICROBIOTEST. Study completion date – September 2, 2009. Laboratory Project Identification Number 320-554.

This study was conducted against Herpes simplex virus type 1 (strain not specified; ATCC VR-260), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Herpes Simplex virus Type 1," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 26 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of newborn calf serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

43. MRID 479579-43 "Virucidal Effectiveness Test, Herpes Simplex virus Type 2, ATCC VR-734" for Carb, F2008.0034, by Tien V. Mai. Study conducted at MICROBIOTEST. Study completion date – September 16, 2009. Laboratory Project Identification Number 320-555.

This study was conducted against Herpes simplex virus type 2 (strain not specified; ATCC VR-734), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Herpes Simplex virus Type 2," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 25 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of newborn calf serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

44. MRID 479579-44 "Virucidal Effectiveness Test Human Coronavirus (strain 229E), ATCC VR-740" for Carb, F2008.0034, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – September 18, 2009. Laboratory Project Identification Number 320-556.

This study was conducted against Human coronavirus (Strain 229E, ATCC VR-740), using MRC-5 cells (ATCC CCL-171) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Human Coronavirus (strain 229E)," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant

mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 10% fetal bovine serum. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 33±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

45. MRID 479579-45 "Virucidal Effectiveness Test Human Influenza B Virus, Charles River Laboratories" for Carb, F2008.0034, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – September 16, 2009. Laboratory Project Identification Number 320-557.

This study was conducted against Human influenza B virus (Strain B/Lee/40; obtained from Charles River Laboratories), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Human Influenza B virus," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 7 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 30 seconds at 20°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of Minimum Essential Medium with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in MEM with 1.0 µg/mL Trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

46. MRID 479579-46 "Virucidal Effectiveness Test Cytomegalovirus, ATCC VR-538" for Carb, F2008.0034, by Tien V. Mai. Study conducted at MICROBIOTEST. Study completion date – October 14, 2009. Laboratory Project Identification Number 320-559.

This study was conducted against Cytomegalovirus (Strain AD-169; ATCC VR-538), using MRC-5 cells (ATCC CCL-171) as the host system. Two lots (Lot Nos. 2008-eg-07 and

2008-eg-08) of the product, Carb, F2008.0034, were tested according to a MicroBioTest protocol titled "Virucidal Effectiveness Test Cytomegalovirus," dated January 19, 2009 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed (3 seconds) with the product at a distance of 6 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 21°C. Five replicates per product lot were tested. Following exposure, the plates were neutralized with an equal volume of fetal bovine serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in DMEM with 5% fetal bovine serum. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 14-21 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were assayed for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

47. MRID 479579-47 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Candida glabrata* (ATCC 2001), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – October 6, 2009. Laboratory Project Identification Number 320-562.

This study was conducted against *Candida glabrata* (ATCC 2001). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.1.04.10.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 40 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Yeast Mold Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 24±2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

48. MRID 479579-48 "AOAC Germicidal Spray Test Supplemental," Test Organism: Penicillin-resistant *Streptococcus pneumoniae* (ATCC 700671), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 11, 2009. Laboratory Project Identification Number 320-563.

This study was conducted against Penicillin-resistant *Streptococcus pneumoniae* (ATCC 700671). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.2.04.10.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 40 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80, 1% Lecithin, and 5% defibrinated sheep's blood to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C and 5% CO_2 candle jar conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Antibiotic resistance of Penicillin-resistant *Streptococcus pneumoniae* (ATCC 700671) was verified on a representative culture. An individual Tryptic Soy Agar plate with 5% defibrinated sheep's blood was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Penicillin-resistant *Streptococcus pneumoniae* (ATCC 700671) to penicillin. See pages 8, 9, and 22 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

49. MRID 479579-49 "Amended Final Report, AOAC Germicidal Spray Test Supplemental," Test Organism: Carbapenem resistant *Klebsiella pneumoniae* (ATCC BAA-1705), for Carb, F2008.0034, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 11, 2009. Amended report date – October 2, 2009. Laboratory Project Identification Number 320-564.

This study was conducted against Carbapenem resistant *Klebsiella pneumoniae* (ATCC BAA-1705). Two lots (Lot Nos. 2008-eg-07 and 2008-eg-08) of the product, Carb, F2008.0034, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995, as reported in Microbiotest protocol 320.1.04.20.09. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed (3 seconds) with the product at a distance of 6-10 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Antibiotic resistance of Carbapenem resistant *Klebsiella pneumoniae* (ATCC BAA-1705) was verified on a representative culture. An individual Mueller Hinton Agar plate was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 10 mm) confirmed antibiotic resistance of Carbapenem resistant *Klebsiella pneumoniae* (ATCC BAA-1705) to imipenum. See pages 9, 16, and 17 of the laboratory report.

Note: The initial report was amended to provide a more complete description of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed. Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. The deviations were reviewed.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Counts (CFU/ carrier)
		Lot No. 2008-eg-07	Lot No. 2008-eg-08	
3-Minute Exposure Time				
479579-01	Community-Associated Methicillin-Resistant *Staphylococcus aureus* Genotype 400	0/10	0/10	1.1×10^5
479579-02	Multidrug-Resistant *Klebsiella pneumoniae*	0/10	0/10	1.9×10^5
479579-03	*Streptococcus pyogenes*	0/10	0/10	2.4×10^4
479579-09	*Burkholderia cepacia*	0/10	0/10	1.2×10^6
479579-10	*Corynebacterium diphtheriae*	0/10	0/10	1.4×10^6
479579-11	*Escherichia coli*	0/10	0/10	5.7×10^4
479579-12	*Enterobacter cloacae*	0/10	0/10	2.3×10^5
479579-13	*Klebsiella oxytoca*	0/10	0/10	8.1×10^5
479579-14	*Listeria monocytogenes*	0/10	0/10	8.8×10^6
479579-15	*Proteus mirabilis*	0/10	0/10	6.5×10^5
479579-16	*Proteus vulgaris*	0/10	0/10	3.3×10^5
479579-17	*Salmonella enterica; serovar Paratyphi B*	0/10	0/10	4.8×10^6
479579-18	*Salmonella typhi*	0/10	0/10	2.2×10^5
479579-19	*Serratia marcescens*	0/10	0/10	2.1×10^5
479579-20	*Shigella dysenteriae*	0/10	0/10	3.5×10^6
479579-21	*Stenotrophomonas maltophilia*	0/10	0/10	8.3×10^6
479579-30	*Campylobacter jejuni*	0/10	0/10	6.9×10^6
479579-31	*Enterobacter aerogenes*	0/10	0/10	2.2×10^5
479579-32	*Enterococcus faecalis*	0/10	0/10	6.1×10^6
479579-33	*Klebsiella pneumoniae*	0/10	0/10	3.3×10^5
479579-34	*Klebsiella pneumoniae* ESBL	0/10	0/10	1.3×10^5
479579-35	*Legionella pneumophila*	0/10	0/10	1.8×10^5
479579-36	*Pseudomonas putida*	0/10	0/10	4.7×10^6
479579-37	*Salmonella enteritidis*	0/10	0/10	2.7×10^5
479579-38	*Streptococcus pneumoniae*	0/10	0/10	7.7×10^4
479579-48	Penicillin-resistant *Streptococcus pneumoniae*	0/10	0/10	7.3×10^4
479579-49	Carbapenem resistant *Klebsiella pneumoniae*	0/10	0/10	1.3×10^6
1-Minute Exposure Time				
479579-24	*Candida albicans* Test Date: 12/13/2008 Test Date: 12/24/2008	 0/10 ---	 1/10 0/10	 1.1×10^4 1.3×10^4
479579-47	*Candida glabrata*	0/10	0/10	1.6×10^5

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 2008-eg-07	Lot No. 2008-eg-08	
30-Second Exposure Time					
479579-04	Human immunodeficiency virus type 1	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{7.93}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{4.80}$	$\leq 10^{4.80}$	
		Log reduction	≥ 3.13 $\log_{10}$	≥ 3.13 $\log_{10}$	
479579-05	Respiratory syncytial virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{5.50}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.00 $\log_{10}$	≥ 3.00 $\log_{10}$	
479579-07	Duck hepatitis B virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.00}$ $FFFUD_{50}$/mL
		10^{-3} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	
		$FFFUD_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-27	Duck hepatitis B virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{5.75}$ $FFFUD_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$FFFUD_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
479579-39	Hantavirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.75}$ $FFFUD_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$FFFUD_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 4.25 $\log_{10}$	≥ 4.25 $\log_{10}$	
479579-42	Herpes simplex virus type 1	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.75}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 4.25 $\log_{10}$	≥ 4.25 $\log_{10}$	
479579-43	Herpes simplex virus type 2	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.50}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 2008-eg-07	Lot No. 2008-eg-08	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 4.00 $\log_{10}$	≥ 4.00 $\log_{10}$	
479579-44	Human coronavirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.25}$ $TCID_{50}$/mL
		10^{-3} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.75 $\log_{10}$	≥ 3.75 $\log_{10}$	
479579-45	Human influenza B virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.60}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.10}$	$\leq 10^{3.10}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-46	Cytomegalovirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{5.75}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
10-Minute Exposure Time					
479579-06	SARS-associated coronavirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.00}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-08	Coxsackievirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.00}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-22	Adenovirus type 14	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{7.55}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 3.75 $\log_{10}$	≥ 3.75 $\log_{10}$	
479579-23	Hepatitis A virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{7.30}$

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 2008-eg-07	Lot No. 2008-eg-08	
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-26	Feline calicivirus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{7.05}$ $TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{2.80}$	$\leq 10^{2.80}$	
479579-28	Feline calicivirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{8.18}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 4.38 $\log_{10}$	≥ 4.38 $\log_{10}$	
479579-29	Rotavirus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{7.00}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.50 $\log_{10}$	≥ 3.50 $\log_{10}$	
479579-40	Adenovirus type 2	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{8.05}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 4.25 $\log_{10}$	≥ 4.25 $\log_{10}$	
479579-41	Echovirus type 12	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$\geq 10^{8.50}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 6.00 $\log_{10}$	≥ 6.00 $\log_{10}$	

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested	
			Lot No. 2008-eg-07, 90 Days	Lot No. 2008-eg-08, 90 Days
5- and 9.5-Minute Exposure Time				
479579-25	*Mycobacterium bovis* BCG Carrier Counts: 1.4×10^4 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product, Carb, F2008.0034, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 3-minute contact time:

Community-Associated Methicillin-Resistant *Staphylococcus aureus* Genotype 400	MRID 479579-01
Multidrug-Resistant *Klebsiella pneumoniae*	MRID 479579-02
Streptococcus pyogenes	MRID 479579-03
Burkholderia cepacia	MRID 479579-09
Corynebacterium diphtheriae	MRID 479579-10
Escherichia coli	MRID 479579-11
Enterobacter cloacae	MRID 479579-12
Klebsiella oxytoca	MRID 479579-13
Listeria monocytogenes	MRID 479579-14
Proteus mirabilis	MRID 479579-15
Proteus vulgaris	MRID 479579-16
Salmonella enterica; serovar Paratyphi B	MRID 479579-17
Salmonella typhi	MRID 479579-18
Serratia marcescens	MRID 479579-19
Shigella dysenteriae	MRID 479579-20
Stenotrophomonas maltophilia	MRID 479579-21
Campylobacter jejuni	MRID 479579-30
Enterobacter aerogenes	MRID 479579-31
Enterococcus faecalis	MRID 479579-32
Klebsiella pneumoniae	MRID 479579-33
Klebsiella pneumoniae ESBL	MRID 479579-34
Legionella pneumophila	MRID 479579-35
Pseudomonas putida	MRID 479579-36
Salmonella enteritidis	MRID 479579-37

Streptococcus pneumoniae	MRID 479579-38
Penicillin-resistant *Streptococcus pneumoniae*	MRID 479579-48
Carbapenem resistant *Klebsiella pneumoniae*	MRID 479579-49

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. Bacteriostasis controls did not show growth.

Note: The "Comments on the Submitted Efficacy Studies" section of this report identifies AOAC method deviations with regard to culture preparation, carrier drying, and subculture incubation. The deviations are overall minor variations from listed temperatures and times, which would be considered standard in a laboratory. Since controls performed as expected, these differences are not considered to have negatively impacted the study validity in this case.

2. The submitted efficacy data support the use of the product, Carb, F2008.0034, as a disinfectant with fungicidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time:

Candida albicans	MRID 479579-24
Candida glabrata	MRID 479579-47

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. [Note that repeat testing was conducted on one product lot (i.e., Lot No. 2008-eg-08) against *Candida albicans*.] Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. When reported, bacteriostasis controls did not show growth.

Note: The "Comments on the Submitted Efficacy Studies" section of this report identifies AOAC method deviations with regard to culture preparation, carrier drying, and subculture incubation. The deviations are overall minor variations from listed temperatures and times, which would be considered standard in a laboratory. Since controls performed as expected, these differences are not considered to have negatively impacted the study validity in this case.

3. The submitted efficacy data support the use of the product, Carb, F2008.0034, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load (a 100% organic soil load for Duck hepatitis B virus) for a 30-second contact time:

Human immunodeficiency virus type 1	MRID 479579-04
Respiratory syncytial virus	MRID 479579-05
Duck hepatitis B virus	MRID 479579-07 and -27
Hantavirus	MRID 479579-39
Herpes simplex virus type 1	MRID 479579-42
Herpes simplex virus type 2	MRID 479579-43
Human coronavirus	MRID 479579-44

Human influenza B virus	MRID 479579-45
Cytomegalovirus	MRID 479579-46

Recoverable virus titers of at least 10^4 were achieved. In studies against all viruses tested at this exposure time, cytotoxicity was observed in the 10^{-2} dilutions. In studies against Human immunodeficiency virus type 1, cytotoxicity also was observed in the 10^{-3} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. [In studies against Duck hepatitis B virus, the initial and confirmatory studies were performed at the same laboratory but under the direction of different study directors.]

4. The submitted efficacy data support the use of the product, Carb, F2008.0034, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10-minute contact time:

SARS-associated coronavirus	MRID 479579-06
Coxsackievirus	MRID 479579-08
Adenovirus type 14	MRID 479579-22
Hepatitis A virus	MRID 479579-23
Feline calicivirus	MRID 479579-26 and 28
Rotavirus	MRID 479579-29
Adenovirus type 2	MRID 479579-40
Echovirus type 12	MRID 479579-41

Recoverable virus titers of at least 10^4 were achieved. In studies against all viruses tested at this exposure time, cytotoxicity was observed in the 10^{-2} dilutions, with one exception: cytotoxicity was not observed in any dilutions in the initial study against Feline calicivirus. In studies against Rotavirus, cytotoxicity also was observed in the 10^{-3} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. [In studies against Feline calicivirus, the initial and confirmatory studies were performed at the same laboratory but under the direction of different study directors.]

5. The submitted efficacy data (MRID 479579-25) support the use of the product, Carb, F2008.0034, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5-minute contact time (also for a 9.5-minute contact time). Complete killing was observed in the subcultures of the required number of carriers against the required number of product lots. No growth was observed in the subcultures of the two extra media. Neutralizer effectiveness testing showed positive growth of the microorganism in Modified Proskauer-Beck Medium, Middlebrook 7H9 Broth, and Kirchner's Medium. Viability controls were positive for growth. Sterility controls did not show growth.

Note: The "Comments on the Submitted Efficacy Studies" section of this report identifies AOAC method deviations with regard to culture preparation, carrier drying, and subculture incubation. The deviations are overall minor variations from listed temperatures and times, which would be

considered standard in a laboratory. Since controls performed as expected, these differences are not considered to have negatively impacted the study validity in this case.

VII RECOMMENDATIONS

A. Regarding submitted data:

1. The proposed label claims that the product, Carb, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 3-minute contact time:

Burkholderia cepacia
Campylobacter jejuni
Carbapenem-Resistant *Klebsiella pneumoniae*
Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA 400)
Corynebacterium diphtheriae
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
ESBL producing *Klebsiella pneumoniae*
Klebsiella oxytoca
Klebsiella pneumoniae
Legionella pneumophila
Listeria monocytogenes
Multidrug-Resistant *Klebsiella pneumoniae*
Penicillin-Resistant *Streptococcus pneumoniae*
Proteus mirabilis
Proteus vulgaris
Pseudomonas putida
Salmonella enterica [*serovar - Paratyphi B*]
Salmonella enteritidis
Salmonella typhi
Serratia marcescens
Shigella dysenteriae
Stenotrophomonas maltophilia
Streptococcus pneumoniae
Streptococcus pyogenes

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Carb, is an effective disinfectant against *Mycobacterium bovis* BCG on pre-cleaned, hard, non-porous surfaces for a 5-minute contact time. This claim is acceptable as it is supported by the submitted data.

3. The proposed label claims that the product, Carb, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 1-minute contact time:

Candida albicans
Candida glabrata

These claims are acceptable as they are supported by the submitted data.

4. The proposed label claims that the product, Carb, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 10-minute contact time:

Adenovirus type 2
Adenovirus type 14
Coxsackievirus B3
Echovirus type 12
Feline calicivirus (surrogate for Norovirus)
Hepatitis A virus
Rotavirus
SARS-Associated Coronavirus (SARS)

These claims are acceptable as they are supported by the submitted data.

5. The proposed label claims that the product, Carb, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 30-second contact time:

Cytomegalovirus
Duck hepatitis B virus (surrogate for Human Hepatitis B virus)
Hantavirus
Herpes simplex virus type 1
Herpes simplex virus type 2
Human coronavirus
Human immunodeficiency virus type 1
Human influenza B virus
Respiratory syncytial virus

These claims are acceptable as they are supported by the submitted data.

6. The following revisions to EPA Form 8570-35 (Data Matrix) are recommended:

- On page 7, change the report identification number for the *Klebsiella oxytoca* study to 320-525.

- On page 10, change the report identification number for the *Streptococcus pneumoniae* study to 320-540

- Add information for the efficacy study against Rhinovirus 39 (ATCC VR-340), an organism identified on the last accepted label and the proposed label.

- Add information for the efficacy study against Poliovirus type 1 (ATCC VR-1562), an organism identified on the last accepted label and the proposed label.

B. Regarding proposed label:

1. On page 3 of the proposed label, delete "no rinse" from the phrase "[This product] is a no rinse disinfectant that disinfects and deodorizes in one labor saving step." Since the product requires a potable water rinse for food contact surfaces, and food contact surfaces are listed on the label, it is inaccurate to state that this is a "no rinse" product.

2. On page 4 of the proposed label, the applicant has added 2009 H1N1 language. New language is in accordance with Agency guidance, and is acceptable. (http://www.epa.gov/oppad001/h1n1-guide.html)

3. The list of surface materials on page 10 of the proposed label lists "painted surfaces" and also identifies "painted surfaces" under the "Do Not Use On" section. This needs to be corrected.

4. The terms "virucidal" and "antiviral" appear throughout the label and are unqualified. The applicant needs to designate these statements with an asterisk that refers back to the organism listing for viruses on page 6.

5. The applicant has made changes to the label to conform with those specified in the Agency's 7/30/09 letter (signed by T. Lantz). These changes are acceptable.

6. The storage and disposal section of the label needs to be revised. It is missing a statement regarding storage in the original container and placement in a locked storage area for household products (PR Notice 83-3). In addition, three optional statements are listed. They do not include the statement, "Non-refillable container. Do not reuse or refill this container," which should be listed on residential use products (the product label lists, "Homes" as a use site).